International Bullous Diseases Group - Consensus on Diagnostic Criteria for Epidermolysis Bullosa Acquisita

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Running head: consensus on EBA diagnostic criteria

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Conflicts of interest:

Enno Schmidt and Zillikens received honoraria for lectures from Roche and Fresenius Medical Care and funding for research and development projects from Euroimmun (ES and DZ), Fresenius Medical Care (DZ), Biotest (DZ), and Novartis (ES). Ron J Feldman receives fees for consulting from Roche. Savas Yayli received fees as principal investigator from Roche. Michael Hertl received honoraria as advisory board from Roche, Janssen, Biogen and Novartis and grants from Biotest, Fresenius and Topas. David T. Woodley hold patents with the University of Southern California (USC) Stevens Institute on various forms of human recombinant type VII collagen, has received grants on EBA and type VII collagen from the National Institutes of Health, Lotus Tissue Repair, Inc., and Shire Pharmaceuticals. He has been a consultant for Biofusion, Lotus Tissue Repair, Inc. and Shire Pharmaceuticals.

What's already known about this topic?

- Currently there is a lack of consensus on the diagnosis of epidermolysis bullosa acquisita (EBA).

What does this study add?

- These recommendations, which have been developed by international experts, provide appropriate pathways for the EBA diagnosis: the algorithms may be used to distinguish EBA from other blistering diseases, which affect the epithelial basement membrane zone.

Summary

Background

Epidermolysis bullosa acquisita (EBA) is a complex autoimmune bullous disease disease with variable clinical presentations and multiple possible diagnostic tests making an international consensus on diagnosis of EBA needed.

Objectives

To obtain an international consensus on the clinical and diagnostic criteria for EBA.

Methods

The international bullous diseases group (IBDG) met three times to discuss the clinical and diagnostic criteria for EBA. For the final voting exercise, 22 experts from 14 different countries voted on 50 different items. When more than 30% disagreed with a proposal, a discussion was held and revoting occurred.

Results

48/50 proposals achieved consensus after discussion. This included 9 diagnostic criteria that are summarized in a flow chart. The IBDG was unable to determine one procedure which would be applicable worldwide

Limitations

Differential diagnosis of bullous systemic lupus erythematosus has not been addressed.

Conclusion

This first international consensus conference established generally agreed upon clinical and laboratory criteria defining the clinical classification and diagnostic testing for EBA. Holding these voting exercises in person with the possibility of discussion prior to voting has advantages in reaching consensus over Delphi exercises with remote voting.

Introduction

The International Bullous Diseases Group (IBDG) was formed in 2005 following a research meeting at the National Institutes of Health for experts in blistering diseases to work towards a consensus for the development and validation of definitions and outcome measures in autoimmune bullous diseases (AIBD).[1] The IBDG focused its efforts initially on pemphigus with consensus definitions, development, and validation of the Pemphigus Disease Area Index (PDAI) and the Autoimmune Bullous Skin Intensity Score (ABSIS).[1,2] Subsequently, the IBDG has published consensus definitions for bullous pemphigoid (BP) and proposed the BP Disease Area Index (BPDAI) severity tool, [3] and then consensus definitions for mucous membrane (MM) pemphigoid (MMP) and the MMP Disease Area Index (MMPDAI).[4] The current project relates to international consensus definitions on diagnostic criteria for epidermolysis bullosa acquisita (EBA).

Two cases of an adult-onset, acquired blistering disease that was reminiscent of patients with hereditary dystrophic epidermolysis bullosa (EB) were reported in 1895 by Elliott.[5] A landmark paper on the subject was in 1971 by Roenigk and colleagues who described three new cases of EBA, reviewed the world literature and proposed the first diagnostic criteria for EBA.[6] These criteria were soon modified by the advent of immunofluorescence (IF) and the finding that all EBA patients had immunoglobulins (Ig)-IgG and sometimes C3 deposits in their dermal-epidermal junction (DEJ) and that by immunoelectron microscopy (IEM), these DEJ immune deposits were clearly in a different localization than immune deposits observed in BP.[7-10] The IgG autoantibodies (auto-Ab) accounting for these DEJ immune deposits were found to be auto-Ab directed against a 290 kDa protein, type VII collagen (Col7), the major component of anchoring fibrils (AFs) in the DEJ.[11,12] Since these initial observations, the diagnostic testing for EBA has undergone significant refinement.[13,15]

Methods

The IBDG met three times during 2015: at the annual meetings of the American Academy of Dermatology in San Francisco (21 experts from 11 countries), the European Society of Dermatological Research in Rotterdam (11 experts from 6 countries), and the European Academy of Dermatology and Venereology in Copenhagen (22 experts from 14 countries). Initial presentations and discussions took place at the first two meetings. After further revisions and discussions at the third meeting, consensus voting took place on the definitions and diagnostic techniques. We defined "consensus" according to Harmonize Outcome Measures for Eczema (HOME) by having less than one-third of the key opinion leaders disagreeing on a given diagnostic criteria.[16,17] Fifty proposals (supplementary table I)

were put forward for discussion and voting related to the diagnostic processes involved in EBA. For each proposal, participants could 'agree', be 'undecided' or 'disagree'. Tables summarizing the literature (supplementary tables II to VI) and examples of cases of EBA (supplementary data 1) were presented for the discussion.

Results

Altogether, 38 experts in EBA from 15 countries took part in this consensus, although not all were able to attend all 3 international meetings held in San Francisco, Rotterdam and Copenhagen. The actual results of the voting questions (supplementary table VII) and the reported anonymized results in the final version of the approved wording or procedures were from 22 blistering disease experts from 14 different countries (Supplementary Fig 1). Although not all members of the IBDG were present at the final voting meeting, all co-authors are concordant with the EBA diagnostic criteria herein.

Definition and clinical variants of EBA

EBA is defined as a subepithelial AIBD in which patients have tissue-bound auto-antibodies (Ab) targeted against Col7 within AFs of the basement membrane zone (BMZ) of DEJ or chorio-epithelial junction (CEJ) in stratified squamous epithelia.

Several forms of EBA exist, with two major types termed: (1) the classical/ mechanobullous form and (2) the non classical/ non mechanobullous forms, first described by Gammon WR *et al* in 1982.[18] The latter includes forms which may clinically resemble BP, MMP, Brunsting-Perry pemphigoid and linear IgA bullous dermatosis (LABD).[6, 18-35] Table I and Figure 1 summarize the descriptions and definitions of these four phenotypes. The

frequency of these subtypes varies in different countries with the classical type being the most common in European reports and the BP-like form more common in Asia (Supplementary Table II).[36-39] It should be recognized that overlapping clinical presentations also may occur (proposal 09, supplementary cases 1 to 5). Overall, because EBA can appear clinically, histologically, and immunologically similar to BP, [18, 20-24] when the initial diagnostic consideration of the dermatologist is BP, EBA should also be considered. Further, if certain clinical clues are somewhat atypical for BP (such as lesions that heal with scarring and milia formation, head and neck involvement, mucosal involvement, disease onset younger than 70), [40] the diagnostic possibility that the patient has EBA, rather than BP, rises considerably. Lastly, it is important to classify MM predominant EBA (MM-EBA) cases because the severity of the mucosal involvement dictates more aggressive, multidisciplinary management.

There has been confusion in the literature as to which forms constituted an 'inflammatory' form of EBA.[13,20,22,23,36-39] The consensus members agreed that the BP-like form is inflammatory but both MM-EBA and IgA-EBA may be inflammatory as well. On the other hand, Brunsting-Perry like EBA is usually a non-inflammatory form of EBA.

Laboratory testing for EBA

The IBDG reached agreement on the following proposal: routine histopathology, direct IF (DIF) microscopy and indirect IF (IIF) that are laboratory tests widely available, allow a diagnosis of subepithelial AIBD but are not able to distinguish EBA from another subepidermal AIBD. Routine histopathology on a biopsy obtained from lesional skin or MM of an EBA patient shows: (i) subepidermal or subepithelial cleavage, (ii) a great variability in the amount or type of inflammatory infiltrate, (iii) milia cysts and fibrosis in older lesions.

Routine DIF microscopy of perilesional skin or MM shows (i) linear immune deposits along the BMZ of DEJ or CEJ and (ii) no labelling of dermal blood vessels. The profile of immune deposits includes IgG and C3 but occasionally IgA or IgM (Supplementary Table III, supplementary case report 5).[41-43] Routine IIF microscopy on monkey, rat or rabbit esophagus or human skin can detect anti-BMZ auto-Ab, but it is often at a low titer.

Currently, the diagnosis of EBA should be confirmed by at least one of the following tests that are only performed in academic centers and are not widely available to the average dermatologist worldwide: [13,15,35] (A) among tests requiring skin or MM biopsies: electron microscopy (EM) and direct IEM,[44] serration pattern analysis by DIF, [41,45,46] and Fluorescent Overlay Antigen Mapping (FOAM), [47-49] and (B) among serological tests for detection of circulating auto-Ab to Col7: ELISA, IIF on BIOCHIP with Col7-NC1 transfected human cells, [50] immunoblotting (IB), IIF on skin deficient in Col7, [51,52] and/ or indirect IEM. [44] All these serological tests obviously require that the EBA patient have auto-Ab to Col7 circulating in their blood and it must keep in mind that anti-Col7 auto-Ab are also present in bullous systemic lupus erythematosus (BSLE).[53]

Alternative laboratory tests when none of these tests is available include: DIF and IIF on SSS,[54] but they will not absolutely clinch the diagnosis of EBA. [55,56]

Standard transmission EM and IEM

In EBA patients, standard transmission EM shows an electron-dense band immediately below the lamina densa (LD) in the AF zone. Another EM finding suggesting EBA is that the cleavage occurs below the LD which remains attached to the roof of the blister. A paucity of AFs also supports the diagnosis of EBA. [44]

Direct IEM on perilesional skin shows *in vivo* bound immune deposits which are very thick, located in the AF zone and a cleavage under immune deposits, if it is present (Fig 3).[44] The IBDG did agree on the limitations of direct IEM (proposal 29). Sixteen members of the consensus group attending the EADV voting had EM available at their site, but only about half of the group had experience in using EM and only 7 were currently using IEM.

Serration pattern analysis.

DIF microscopy of a perilesional skin biopsy can distinguish EBA from other sub-epithelial AIBD by showing an u-serrated linear pattern of Ig deposits along the BMZ in EBA and BSLE and a n-serrated pattern of Ig deposits in BP, anti-laminin 332 MMP and anti-p200/laminin γ1 pemphigoid (Fig 2).[41,45,46,55,56] No agreement was obtained on limitations of the serration pattern analysis (proposal 26). Indeed it can be performed with routine DIF microscopy (new unpublished data on exact requirements in supplementary data 2). However, to date this test is not widely available and only the teams in Groningen and Lubeck have been able so far to master the technique (proposal 26bis).

Fluorescent Overlay Antigen Mapping analysis

FOAM, using routine IF microscope with either an image analysis system[47] or a laser scanning confocal microscopy[48,49], shows in EBA *in vivo* bound immune deposits below basal keratinocytes membrane, lamina lucida and LD components (Fig 4).

ELISA

Commercially available ELISAs using recombinant NC1/NC2-Col7, [57-60] or NC1-Col7,[50] are widely available. Sensitivities vary depending on the selection criteria. It is very high (79-

96.7%) on preselected positive sera by IIF on SSS, with floor labelling (Supplementary Table IV). The sensitivity of ELISA to NC1/NC2 is lower (30-54%) in studies on unselected EBA sera.[39, 61]

ELISA using a full-length Col7 is more sensitive than ELISA using NC1/NC2-Col7 but not commercially available (Supplementary Table V).[39,42,62,63]

ELISA Col7 are not highly specific since they may be positive in patients with Crohn's disease or ulcerative colitis without cutaneous manifestations of EBA,[64] atypical AIBD,[65] and patients with recessive dystrophic EB (RDEB) (Supplementary Tables V, VI). [39,63,66,67] Of note, the presence of circulating auto-Ab against BP180, laminin 332, the p200/laminin γ1chain (detected by IB or ELISA), which may occur due to the epitope spreading phenomenon, does not rule out the diagnosis of EBA (Supplementary cases 2 and 4).[37,68-75]

Novel technique: IIF microscopy using Col7-NC1 transfected cells

Patient serum autoAb could label Col7-NC1 transfected cells on a special slide, a so-called BIOCHIP^{TM} method (Fig 5). This test is now commercially available and could be used as a substitute for the ELISA outlined above. It is a sensitive, specific and rapid assay for testing preselected positive sera by IIF on SSS, with floor labelling.[50] Like serological testing by ELISA, its sensitivity is lower in studies on unselected EBA sera.[39]

Immunoblotting

IB is a serological technique performed on extracts of tissues or cells or recombinant Col7 proteins (Fig 6).[13,15,35] IB is effective for diagnosing EBA by detecting auto-Ab in patient

sera that labels the Col 7 α-chain. IB can substitute for the commercially available ELISA. The IBDG did agree on the limitations of IB (proposal 33).

IIF on Col7-deficient human skin and indirect IEM

A definitive diagnosis of EBA can be demonstrated by IIF when presumptive EBA sera that label the DEJ of normal human skin, but do not label skin from generalized severe RDEB with absent Col7 (Fig 7).[51,52] Obviously, this is not offered in commercial laboratories and is limited by the access to skin specimens from patients with exceedingly rare diseases.

A definitive diagnosis of EBA can also be demonstrated when presumptive EBA sera label the AFs by indirect IEM (Fig 3).[44]

DIF and IIF microscopy on SSS

DIF and IIF on SSS are alternative laboratory tests which only give a probable diagnosis of EBA. DIF is performed after splitting the skin biopsy of the patient using 1M NaCl (Supplementary data 3).[54,76] It is not possible with MM biopsies. IIF may be performed on either normal human SSS using a similar procedure, [119,77-79] or monkey SSS, which is commercially available. Immune deposits in EBA patients remain on the dermal floor of the separation, while BP immune deposits remain with the epidermal roof (Fig 8). However, this dermal labelling is not specific to EBA. It is also seen in anti-laminin 332 MMP and anti-p200/ laminin γ1 pemphigoid (Fig 4). Additional tests are necessary to exclude reactivity against these molecules and finally diagnose an EBA. Overall, IIF on SSS is more sensitive than IIF on monkey or rat oesophagus or unsplit human skin for detecting anti-BMZ auto-Ab.[77,79]

Lastly, the IBDG updated consensus criteria for EBA diagnosis (2015) include combinations of the following tests:

1. A bullous disorder within the defined clinical spectrum
2. Histopathology revealing a subepidermal or subepithelial blister
3. A positive DIF microscopy of perilesional skin or MM with linear IgG, C3, IgA and/or IgM deposits within the epithelial BMZ
4. Detection of circulating auto-Ab against Col7 by IB, ELISA and/or IIF microscopy on Col7-expressing human cells
5. Labelling AFs by indirect IEM or negative IIF microscopy on Col7-deficient skin
6. An u-serration pattern by DIF microscopy
7. Direct IEM of perilesional skin demonstrating immune deposits within AFs zone +/- the lower LD.
8. *In vivo* bound immune deposits below type IV collagen by FOAM
9. Alternatively to item 4 to 8, dermal labelling by DIF and/or IIF on SSS

The IBDG did reach agreement that the ideal scenario is for a putative EBA disease patient (criteria 1) to exhibit a sub-epidermal bulla by histology (criteria 2 optional), a positive DIF microscopy (criteria 3), and an ELISA (or another serological test) showing that the patient's serum auto-Ab targets col7 (criteria 4 or 5). In this scenario, a highly probable diagnosis of EBA has been made and no further tests need to be done for confirmation. Unlike this ideal scenario for the diagnosis of EBA, the problem that often arises[39, 61] is when an EBA patient lacks circulating auto-antibodies and therefore IIF, SSS IIF and ELISA are negative. Then the diagnosis of EBA could be considered definitive if items 1 and 3 and at least one item between items 6 to 8 are present (item 2 is optional). Lastly if tests 6-8 could

not be done, the diagnosis of EBA is possible if items 1, 3 and 9 are present; then diagnosis has to be confirmed by exclusion of auto-immunity against laminin 332 or the p200/laminin γ1 chain. The consensus conference was not able to determine one procedure which would be applicable for the whole world. There was an animated discussion about what should be considered routine (Proposal 18). Which test(s) that the practitioner chooses will likely be determined by the clinical presentation of the patient (classical/mechanobullous type or not), the geography of the practitioner and which test is most logistically accessible. Figure 9 is a flow diagram summarizing the different diagnostic investigative pathways.

In conclusion, this consensus of the criteria for the diagnosis of EBA provides a general framework for establishing the diagnosis of EBA and takes into account the clinical presentation and available laboratory testing. These criteria should help clinicians from mis-diagnosing other AIBDs and missing the diagnosis of EBA. They will also be useful for future studies designed to define the natural history and therapeutic outcomes of EBA.

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Figure Legends

Figure 1. Clinical subtypes of epidermolysis bullosa acquisita (EBA). A-G, Classical / mechanobullous form of EBA: trauma induced lesions are preferably localized to the extensor skin surfaces *i.e* dorsal hands (A, B), feet (C), heel (D), elbows (E) and knees (F). Bullous/vesicular lesions or erosions are surrounded by non-inflamed or scarred skin (B, D). Lesions heal with scarring (E, F) and milia formation (A, B, F). Nail dystrophy (C) and scarring alopecia (G) are possible. H-J, bullous pemphigoid (BP)-like form of EBA: tense bullae on erythematous (H) or urticarial skin (I, J) suggestive of BP, in atypical locations for BP at extensor areas of the limbs (H-J). K-P, Mucous membrane (MM) EBA: all the MM lined by squamous epithelium may be involved, particularly the tongue in buccal MM (K), anus (L), esophagus (M) leading to strictures and gastrostomy (N), conjunctival scarring (O), tracheal

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Figure 2. Serration pattern analysis by direct immunofluorescence. The u-serrated pattern is characterized by closed arches at the bottom appearing like "growing grass", while the n-serrated pattern shows closed arches at the top. A, direct immunofluorescence of epidermolysis bullosa acquisita skin showing linear IgG deposition along the epidermal BMZ in an *u-serrated* pattern (400x). B, direct immunofluorescence of bullous pemphigoid skin showing linear IgG deposition along the epidermal BMZ in a *n-serrated* pattern (400x). *Courtesy of Dr Gilles Diercks.*

Figure 3. Immunoelectron microscopy (IEM) in epidermolysis bullosa acquisita. A, direct IEM in pre-embedding immunoperoxidase technique. Thick immune deposits are observed in the anchoring fibril zone below the lamina densa (LD) and split (*) under them. B and C, indirect IEM in respectively pre-embedding immunoperoxidase and immunogold technique. Immune

deposits (arrows) decorate the ends of anchoring fibrils. *2A, 2B and 2C = Fig. 19.10b, 19.12a and 19.12b in Prost–Squarcioni C. Electron microscopy and immunoelectron microscopy, in Murrell D ed. Blistering Disease: Clinical Features, Pathogenesis, Treatment. New York, NY: Springer 2015, with the permission of Springer*

Figure 4. Fluorescent overlay antigen mapping (FOAM) in skin biopsies. In epidermolysis bullosa acquisita (EBA), *in vivo* bound immune deposits are below the $\alpha 6\beta 4$ integrin of the basal keratinocytes membrane and below the components of the lamina lucida and the lamina densa (laminin 332 and type IV collagen). A, in a patient with EBA, in vivo bound IgG (green) are below type IV collagen (red). B, in a patient with a bullous pemphigoid, in vivo bound IgG (green) are above laminin 332 (red). C, in a patient with mucous membrane pemphigoid (MMP), in vivo bound IgG (green) are below or colocalize (yellow) with laminin 332 (red). D, in a patient with MMP, in vivo bound IgG (green) are above or colocalize (yellow) with type IV collagen (red) in *D. Courtesy of Dr Katarzyna Wozdniak*

Figure 5. Indirect immunofluorescence microscopy with NC1 type VII collagen (NC1-Col7)-expressing human cells by BIOCHIP™ technology**.** Patient serum autoAb could label molecularly engineered epidermal cells that express human NC1-Col7 on a special slide. A, on a standard-sized slide, there are five incubation fields each with two different BIOCHIPs: one with HEK293 cells transfected with pTriEx-1 which serve as negative control and one with human HEK293 cells transfected with NC1-Col7. B, autoantibodies in the serum of a patient with epidermolysis bullosa acquisita labeled NC1-Col7expressing HEK293 cells *(right image)* but not non NC1-Col7expressing cells *(left image)*. C, no reactivity of both NC1-Col7 expressing and non NC1-Col7 expressing cells is seen with normal human serum*. Courtesy of Dr Aucouturier, Paris.*

Figure 6. Immunoblotting (IB) in epidermolysis bullosa acquisita (EBA). By immunoblotting with dermal extract, EBA serum recognized a band at 290 kDa which is the alpha chain of type VII collagen (Col7), different from laminin γ1/ p200 protein. A second band of 145 kDa which is the amino-terminal NC1 domain of the alpha chain of Col7 could be seen. NHS, normal human serum.

Figure 7. Indirect immunofluorescence (IF) microscopy on type VII collagen (Col7)-deficient skin versus normal skin. A, indirect IF microscopy with epidermolysis bullosa acquisita (EBA) serum on Col7 containing normal human skin showing positive IgG binding along the epidermal basement membrane zone (BMZ) (400x). B, indirect IF microscopy with EBA serum on Col7 knockout skin from patient with severe generalized recessive dystrophic epidermolysis bullosa showing negative IgG binding along the epidermal BMZ (400x). *Courtesy of Dr Hendri Pas, Groningen*

Figure 8. Indirect immunofluorescence microscopy on salt-split skin. An artificial cleavage is induced by NaCl 1M in monkey skin (A, B, counterstained by Evans blue *Courtesy of Dr Aucouturier, Paris.*) or normal human skin (C, D). A, C: labelling of the floor of the cleavage by the serum of a patient with EBA. B, D: labelling of the roof of the cleavage by the serum of a patient with bullous pemphigoid.

Figure 9:

Flow chart for diagnosis of epidermolysis bullosa acquisita (EBA). DIF, direct immunofluorescence. AIBD, autoimmune blistering disease. Ig, immunoglobulin. DEJ, dermo-epidermal junction. CEJ, chorio-epithelial junction. Auto-Ab, auto-antibodies. Type

VII collagen (Col7) specific ELISAs include NC1, NC1/NC2 or full length Col7 ELISAs. The BIOCHIP™ technology uses NC1 Col7-transfected HEK293 cells as substrate for indirect immunofluorescence (IIF) microscopy (IIFT test). WB, Western blotting. Col7-deficient skin is obtained from patients with generalized severe recessive dystrophic epidermolysis bullosa (RDEB). MM, mucous membranes. IEM, immunoelectron microscopy. FOAM, fluorescent overlay antigen mapping. LL, lamina lucida. LD, lamina densa. BP, bullous pemphigoid. MMP, mucous membrane pemphigoid. IF, immunofluorescence. SSS, salt split skin: an artificial cleavage is obtained by incubation of normal monkey or human skin with NaCl 1M. LN, laminin.*, NC1 ELISA, NC1/NC2 ELISA, NC1 Col 7-transfected HEK293 cells and monkey SSS are commercially available. † Negativity on Col7 deficient skin is significant if IIF microscopy on normal human skin is positive. ‡ include WB with dermal extract or recombinant C-terminus LN gamma 1 or IIF on LN 332 deficient skin.

Appendix

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Table I: definitions of clinical forms of EBA

Classical/mechanobullous

One subtype only[6]

characterized by

- trauma induced lesions (skin fragility)*
- bullous/vesicular lesions or erosions
- encompassed by non-inflamed or scarred skin.
- scarring* and milia formation*
- preferably localized to trauma-prone sites and the extensor skin surface (dorsal hands*, elbows*, knees*, Achilles tendon, feet*).
- possible nail dystrophy*
- possible scarring alopecia

Non classical/ non mechanobullous

BP- like EBA[18, 20-24]

defined as an eruption

- with features characteristic of BP (pruritus, tense bullae on erythematous or urticarial skin, involvement of trunk and folds)
- usually mixed with atypical lesions for a BP (skin fragility, bullae on normal skin, milia, involvement of face or extensor area of the limbs).

MM-EBA[20-27]

defined as a disease that predominantly affects MM lined by squamous epithelium, i.e MM of

- mouth,
- pharynx,
- oesophagus,
- epiglottis,
- conjunctiva,
- genitalia,
- anus

and respiratory tract in malpighian metaplasia

Brunsting-Perry type EBA[28-32]

defined as a chronic recurrent blistering dermatosis confined to the head and neck

IgA-EBA[20, 33, 34]

defined as a disease that presents with linear IgA deposits in the BMZ that can be observed by direct IF

- it may resemble LABD
- it may be more aggressive with mucosal scarring

EBA = epidermolysis bullosa acquisita, BP = bullous pemphigoid, MM = mucous membranes, * = criteria of Roenigk, BMZ = basal membrane zone, IF = immunofluorescence, LABD = linear IgA bullous dermatosis

Fig 1. Clinical subtypes of epidermolysis bullosa acquisita (EBA).

A-G, Classical / mechanobullous form of EBA: trauma induced lesions are preferably localized to the extensor skin surfaces *i.e* dorsal hands (A, B), feet (C), heel (D), elbows (E) and knees (F). Bullous/vesicular lesions or erosions are surrounded by non-inflamed or scarred skin (B, D). Lesions heal with scarring (E, F) and milia formation (A, B, F). Nail dystrophy (C) and scarring alopecia (G) are possible.
H-J, bullous pemphigoid (BP)-like form of EBA: tense bullae on erythematous (H) or urticarial skin (I, J) suggestive of BP, in atypical locations for BP at extensor areas of the limbs (H-J).

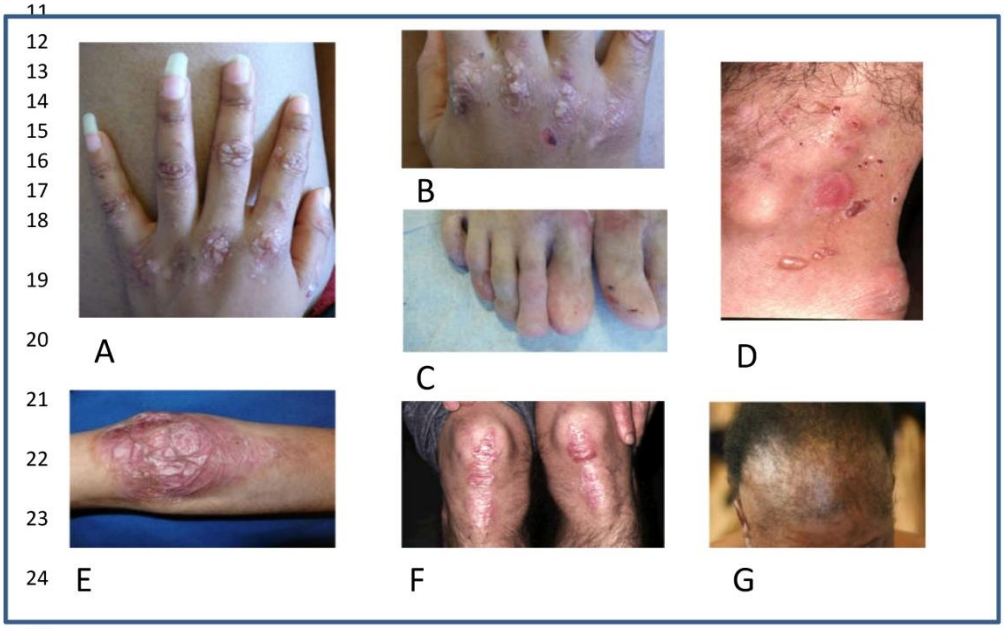
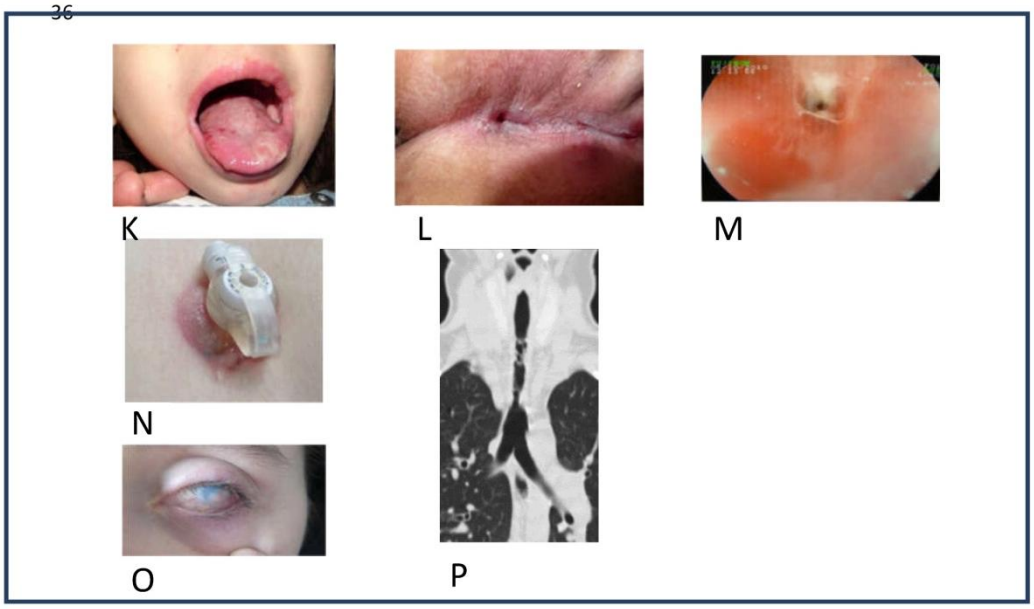


Fig 1 (continued).

K-P, Mucous membrane (MM) EBA: all the MM lined by squamous epithelium may be involved, particularly the tongue in buccal MM (K), anus (L), esophagus (M) leading to strictures and gastrostomy (N), conjunctival scarring (O), tracheal stenosis (P).
Q, IgA EBA with bullous eruption in strings of pearls.
R, Brunsting-Perry type EBA with recurrent blistering dermatosis confined to the head for ten years.



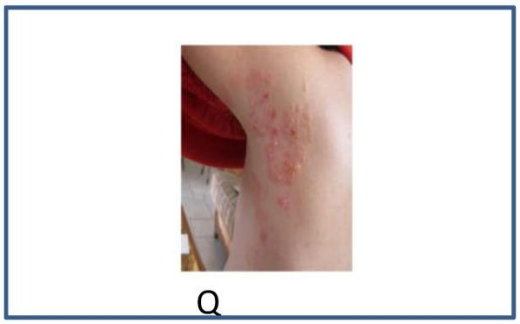
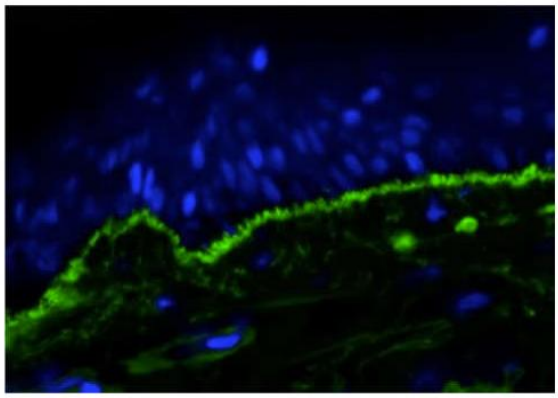


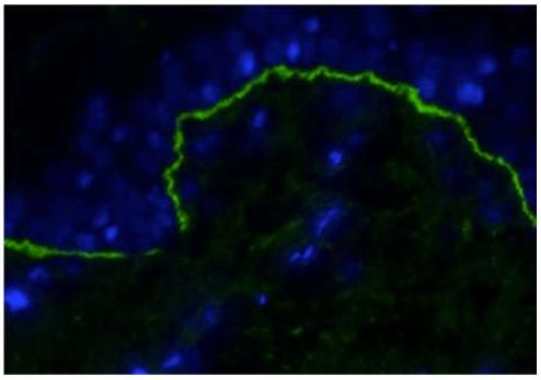
Fig 2. Serration pattern analysis by direct immunofluorescence.

A u-serrated pattern is characterized by closed arches at the bottom appearing like "growing grass", while the n-serrated pattern shows closed arches at the top

A. Direct immunofluorescence of epidermolysis bullosa acquisita skin showing linear IgG deposition along the epidermal BMZ in an *u-serrated* pattern (400x)



B. Direct immunofluorescence of cutaneous pemphigoid skin showing linear IgG deposition along the epidermal BMZ in a *n-serrated* pattern (400x)



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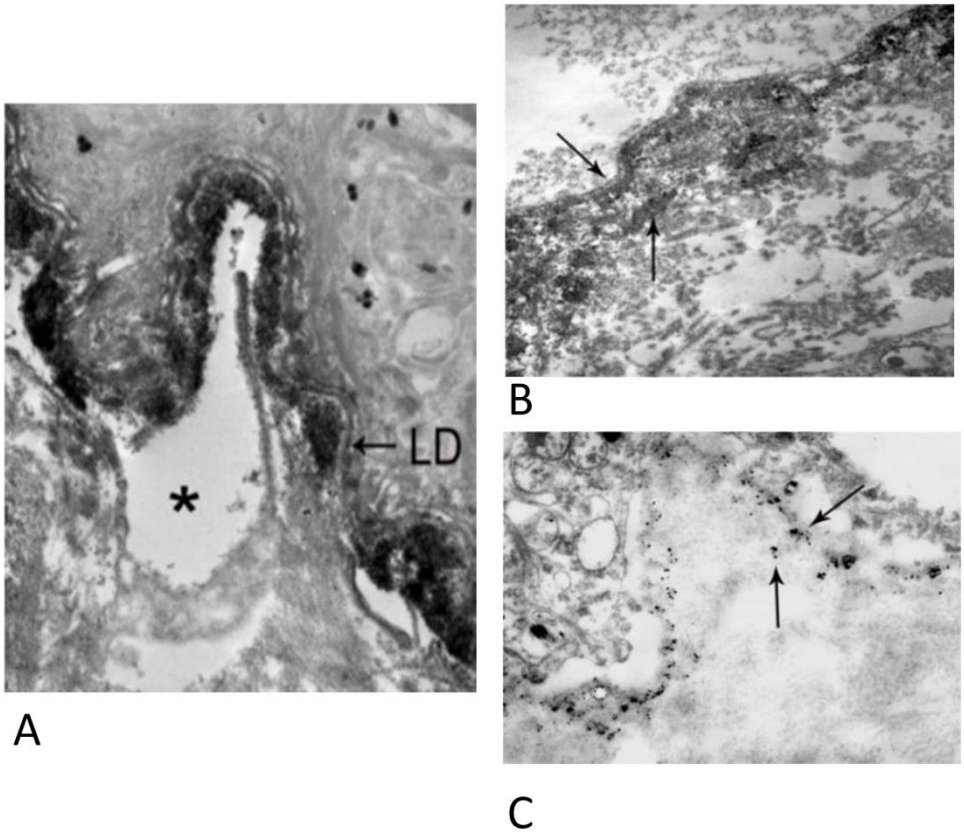
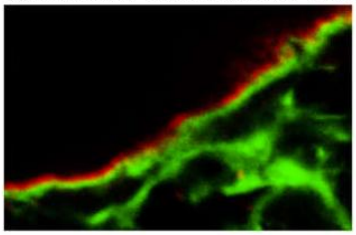


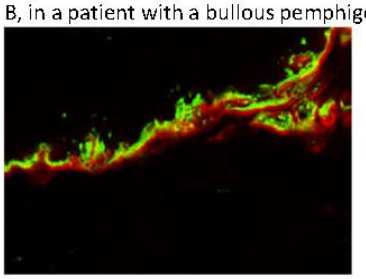
Fig 4. Fluorescent overlay antigen mapping (FOAM) in skin biopsies.

In EBA *in vivo* bound immune deposits are below the α6β4 integrin of the basal keratinocytes membrane and below the components of the lamina lucida and the lamina densa (laminin 332 and type IV collagen). *Courtesy of Dr Katarzyna Wozdniak*

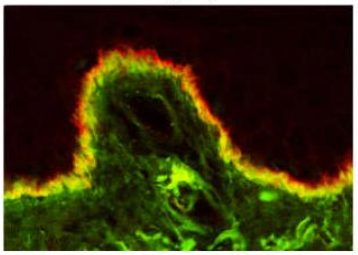
A, in a patient with EBA, in vivo bound IgG (green) are below type IV collagen (red).



B, in a patient with a bullous pemphigoid, in vivo bound IgG (green) are above laminin 332 (red).



C in a patient with mucous membrane pemphigoid, in vivo bound IgG (green) are below or colocalize (yellow) with laminin 332 (red)



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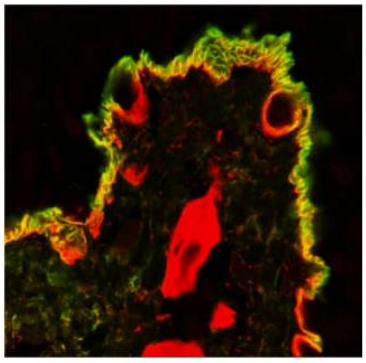

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Patient serum autoAb could label molecularly engineered epidermal cells that express human NC1-Col7 on a special slide.
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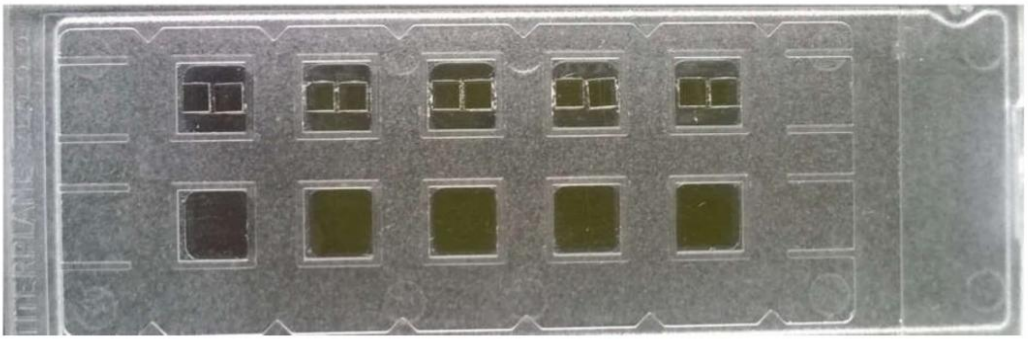

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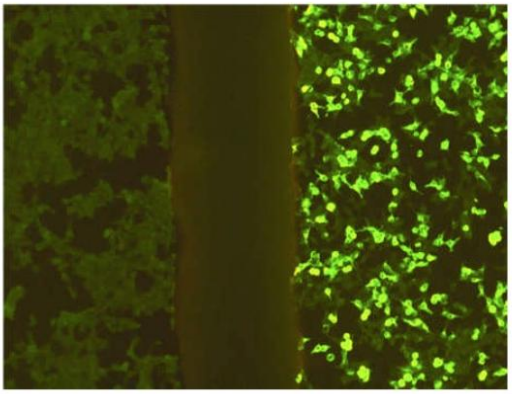

B C

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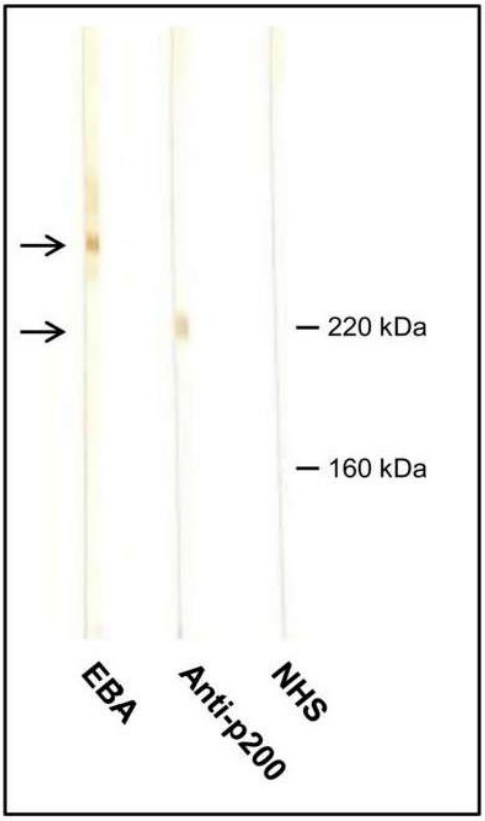

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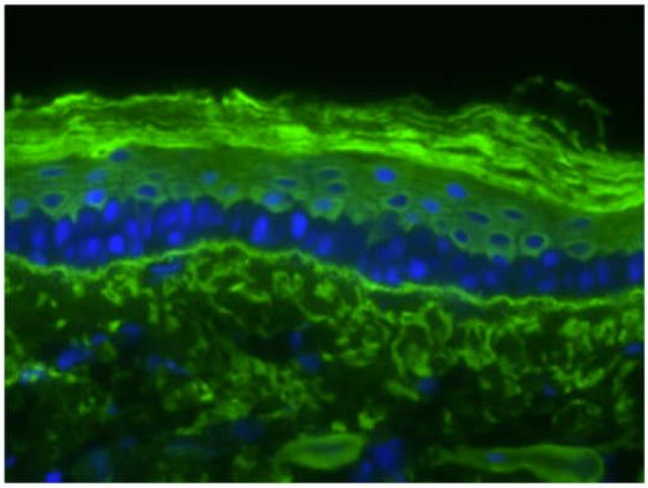
A. IIF with epidermolysis bullosa acquisita (EBA) serum on Col7 containing normal human skin showing positive IgG binding along the epidermal BMZ (400x) *Courtesy of Hendri Pas, Groningen*



B. IIF with EBA serum on **Col7 knockout skin** from patient with severe generalized recessive dystrophic epidermolysis bullosa showing negative IgG binding along the epidermal BMZ (400x). *Courtesy of Hendri Pas, Groningen*

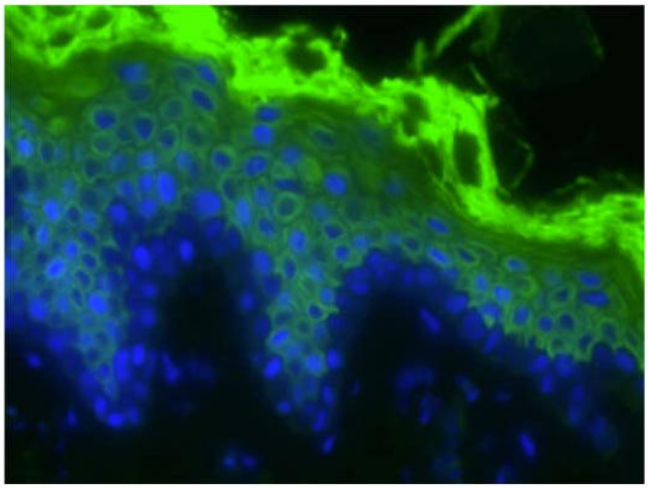
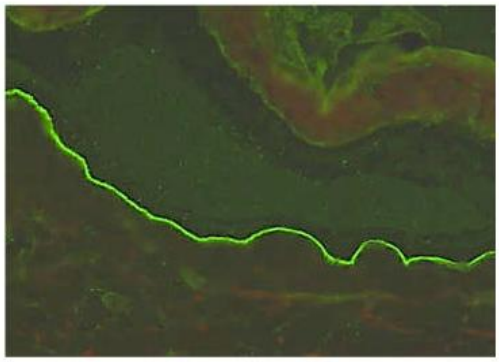


Fig 8. Indirect immunofluorescence on salt split skin.

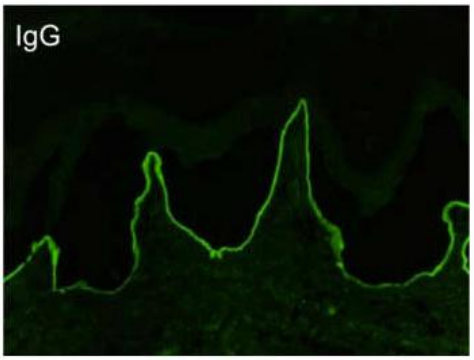
An artificial cleavage is induced by NaCl 1M in monkey skin (A, B, counterstained by blue Evans *Courtesy of Dr Aucouturier, Paris)* or normal human skin (C, D).

A, C: labelling of the floor of the cleavage by the serum (IgG) of a patient with EBA.

A

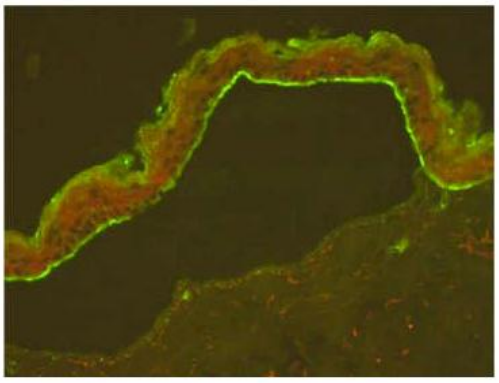


C

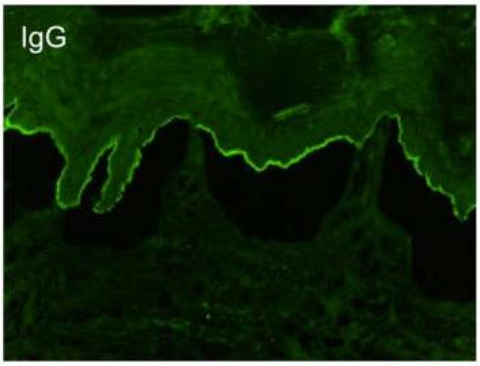


B, D: labelling of the roof of the cleavage by the serum of a patient with bullous pemphigoid.

B



D



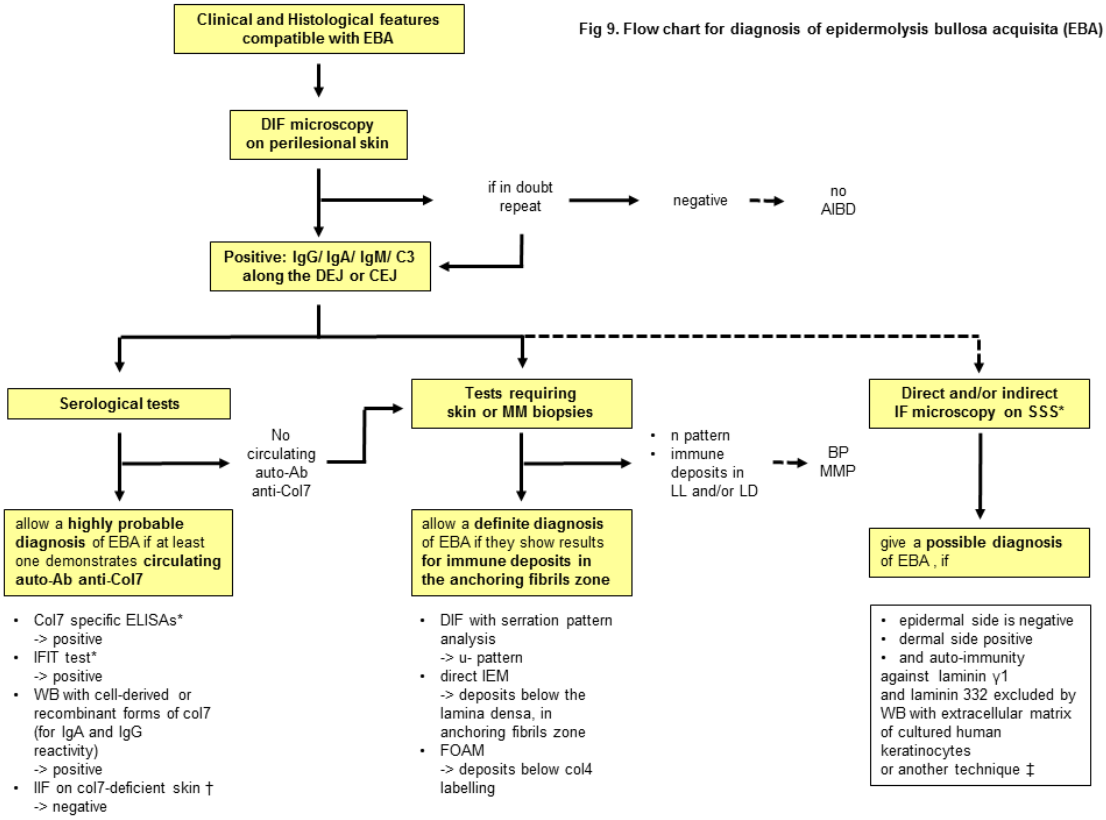


Fig 9. Flow chart for diagnosis of epidermolysis bullosa acquisita (EBA)